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Introduction*

by John F. Rosen,† Raymond F. Novak,‡ and Michael J. Galvin§

The ultimate thrust that serves as the basis for biological research is the need to understand the mechanism(s) that underline biological events. For example, over the years many theories have been advanced to explain the mechanism(s) of ischemia-induced cardiac injury. Subsequently, based on the results of numerous studies that supported or disproved mechanistic theories for ischemic injury, new pharmacological agents have been developed for meliorating the ischemic injury by blocking one or more of the mechanisms, e.g., calcium influx leading to injury. Similarly, the mechanism(s) by which environmental agents cause adverse health effects are of great interest. In general, many diseases affect primarily a single organ system, whereas environmental agents adversely affect a number of organ systems simultaneously. For example, certain pesticides have been shown to affect the central and peripheral nervous, reproductive, and digestive systems. Understanding the mechanism(s) of environmental agent-mediated toxicity is complicated further by the fact that some chemicals are intrinsically toxic whereas others require metabolic conversion to toxic products in the body. Through our understanding of the processes by which environmental agents affect human health, an agressive and effective program for understanding, ameliorating, and preventing environmentally induced disease can be developed.

Although an environmental agent can affect a variety of organ systems, there is evidence that similar mechanisms may be involved in the cellular response to a toxic insult regardless of the organ system affected. The calcium ion has been implicated as a possible common mediator of many important physiological and pathophysiological processes. A recent symposium was held in order to address the significant and complicated role of the complex calcium messenger system.

Physiologic and Pathologic Roles in Calcium

Recent developments and wide applications of the techniques for measuring intracellular free calcium $[Ca^{2+}]_i$ have been accompanied by rapid advances in understanding the central and diverse roles of $[Ca^{2+}]_i$ in the modulation of cell function. Initially, most of the information concerning the calcium messenger system was obtained from studies of nerve and muscle tissues, which provided an early understanding of how events in the cytosol are regulated by rapid changes in the concentration of $[Ca^{2+}]_i$. The effects of $[Ca^{2+}]_i$ on cardiac contractility were rapidly turned into relevant clinical terms, as calcium channel blockers are now widely used in the management of patients with heart disease. Effects of $[Ca^{2+}]_i$ on smooth muscle regulation have also been translated into therapeutic regimens and a pathogenetic role for $[Ca^{2+}]_i$ in essential hypertension.

More recently, the complexity and diversity of the calcium messenger system in controling multiple cell functions have been expanded considerably to include inositol lipid metabolism, inositol phosphate turnover, calcium homeostasis, and protein phosphorylation (*1,2*). The calcium messenger system, which transduces extracellular events into intracellular ones, now includes a far more finely orchestrated cascade of events than those first described in nerve and

*Organizing committee: Faye Calhoun, Division of Research Grants, National Institutes of Health, Bethesda, MD; Michael J. Galvin, Jr., Division of Extramural Research and Training, National Institute of Environmental Health Sciences, Research Triangle Park, NC; Doyle Graham, Department of Pathology, Duke University, Durham, NC; Richard Irons, Biochemical Toxicology and Pathobiology, Chemical Industry Institute of Toxicology, Research Triangle Park, NC; Raymond F. Novak, Institute of Chemical Toxicology, Wayne State University, Detroit MI.

†Department of Pediatrics, Albert Einstein College of Medicine, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467.

‡Institute of Chemical Toxicology, Wayne State University, 2727 Second Avenue, Detroit, MI 48210.

§National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

Address reprint requests to J. Rosen, Department of Pediatrics, Albert Einstein College of Medicine, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467.

skeletal muscle. Recently gained knowledge indicates that this complex system also includes the turnover of plasma membrane phosphatidylinositides, which give rise to other transducing messengers, such as inositol triphosphate and diacylglycerol. These more recently identified messengers bring about the activation of plasma membrane-associated protein kinase C that mediates, via an increase in calcium cycling across the plasma membrane, sustained cellular responses (*1,2*). Such sustained cellular responses involve modulation of calcium channels and/or influx of calcium as a direct result of hormone-receptor complexes. As this expanded knowledge has emphasized the central role of the calcium messenger system in cell activation, disorders of this system involve an enlarging array of disease states, including mania, depression, bronchial asthma, varous endocrinopathies, clotting abnormalities, and the adult onset of diabetes.

Given the remarkable complexity and unique diversity of the calcium messenger system, demonstration of direct and selective effects of toxicants on intracellular calcium homeostasis and calcium-mediated cellular processes is likely to be a highly challenging effort for toxicologists (*3*). It may prove to be quite difficult for toxicologists to demonstrate specific mechanisms of toxicity localized to sites within the calcium-messenger system. There may be insufficient reason, *a priori*, to suggest that a highly specific mechanism of toxicity exists; several critical intracellular events may be perturbed concurrently, with toxicity being the net summation of a number of such changes. Frequent and careful assessments are required by toxicologists to ascertain which observations are mechanistic and worthy of detailed investigation, compared to those that are epiphonomena. Nonetheless, well-characterized quantitaive, functional, and mechanistic studies of discrete and selective toxicant effects on the calcium messenger system may yield new and fundamental information in toxicological reasearch. This appears to be an ever-intriguing path of investigation by toxicologists. The path is likely to be challenging conceptually and methodologically, but one that holds considerable promise and potential for further understanding molecular targets of toxicity at the cellular level.

Methods of Monitoring Measuring Calcium Levels

In order to understand the diverse roles of the calcium messenger system, substantial emphasis has been directed towards characterizing the components of the calcium messenger system, the cytosolic-free calcium levels, and the effects of alterations in cytosolic-free calcium on cellular function. As with any scientific endeavor, a fundamental question arises as to which approach or technique should be employed to assess accurately, within a given system, alterations in cytosolic-free calcium. The session that addressed methodological approaches to measure cytosolic-free calcium was intended to provide the audience with an overview of different techniques, applicable systems, and appropriate limitation.

The uses and limitations associated with various indicators such as Quin 2; Fura-2; and the photoproteins, of which aequorin is prototypic, were described. Of these, Fura-2 appears to be the most promising; whereas the use of Quin 2 may be limited by a high affinity for calcium. Although the photoproteins have been employed successfully as Ca^{2+} indicators in a variety of different cell types, the greatest use has been in muscle cells. Difficulties associated with photoprotein indicators include introduction of the large molecules into cells, the modest response rate of the indicators to sudden changes in Ca^{2+} levels, anatagonism of Ca^{2+} effects by Mg^{2+}, and linearity between Ca^{2+} concentration and light intensity.

Multiparameter digitized video microscopy (MDVM) is a new, developing methodologic approach that can be employed for examining simultaneously multiple, specific cellular parameters of interest at the single cell level. Changes in cytosolic-free Ca^{2+}, mitochondrial membrane potential, cytosolic pH, and cell surface blebbing can be monitored through the use of multiple fluorescent probes having excitation and emission spectra that respond to a singular parameter. The fluorescence images for each probe are collected as a function of time, digitized, and stored. This approach is unique in that it should allow resolution of the temporal sequence of events that ultimately leads to irreversible cell injury. The major limitations would appear to be development of the instrumentation required, specificity of the fluorescent probes for the desired parameter, accessibility of the probe to the cell or to the subcellular compartment, and potential complications arising from the simultaneous use of multiple probes.

Another exciting and novel approach for monitoring intacellular Ca^{2+}, involves the application of ^{19}F nuclear magnetic resonance (NMR) in conjunction with the fluorinated calcium ion chelator 5F-BAPTA (1,2 bis(2-amino-5-fluorophenoxy) ethane *N,N,N′,N′*-tetraacetic acid). The use of fluorinated ion chelators permits the determination of cytosolic free calcium levels *in situ*. This approach will also allow investigation of the temporal relationship between elevation of cytosolic calcium levels and lethal cell injury. An additional advantage associated with use of fluorinated calcium chelators is the absence of interference from other metal ions. Thus, studies on alterations of free calcium levels and related kinetic processes produced by other metal ions, such as Pb^{2+}, can be readily performed since separate NMR signals can be distinguished for the fluorinated chelator when it is bound to other metals. Indeed, the use of these fluorinated chelators permits the simultaneous measurement of Ca^{2+} and other metal ions such as

Pb^{2+}, thereby providing a method for characterizing the effects of other heavy metals, which may normally interfere with calcium measurements on cellular calcium homeostasis.

The limitations of this approach are those traditionally associated with the use of magnetic resonance spectroscopy and include sensitivity and data acquisition time. Nonetheless, the ability to monitor changes in cellular Ca^{2+} levels in perfused organ, in individual cells, or *in vitro* without interference from background contributions or other metal ions is unique and represents a significant strength of this analytical approach.

Proceedings of the Conference

The purpose of this conference was to bring together scientists, from many different disciplines, who are interested in the physiological and pathological roles of calcium. To this end the National Institute of Environmental Health Sciences hosted a conference on March 7 and 8, 1988, which covered a wide spectrum of topics concerning the biological roles of calcium, methods of measuring calcium changes, and the roles of calcium in the specific cellular events. It is hoped that the conference and the publication of its proceedings will provide a resource for investigators interested in examining the role of calcium in environmental agent toxicity. It is also anticipated that other investigators who are working with appropriate biological models will focus some of their expertise on the problem of environmental agent toxicity.

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